A TAXONOMIC STUDY OF THE GENUS *LAURENCIA* (CERAMIALES, RHODOPHYTA) FROM VIETNAM. I. *LAURENCIA CADUCIRAMULOSA* MASUDA *ET* KAWAGUCHI, SP. NOV.

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ABSTRACT — *Laurencia caduciramulosa* Masuda *et* Kawaguchi, sp. nov. (Ceramiales, Rhodophyta) is described from Vietnam. It is characterised by the presence of four vegetative periaxial cells per axial segment, longitudinally oriented secondary pit-connections between contiguous superficial cortical cells projecting at the apices of branches, lenticular thickenings in the walls of medullary cells, and dwarf, deciduous branchlets that may function as propagative organs (propagules). This species is also characterised by the following set of halogenated secondary metabolites: aplysiadiol (diterpenoid), deoxyprepacifenol (sesquiterpenoid), laurenenyne-A and laurenenyne-B (C_{15} acetogenins).

RÉSUMÉ — *Laurencia caduciramulosa* Masuda *et* Kawaguchi, sp. nov. (Ceramiales, Rhodophyta) est décrite du Vietnam. L'espèce est caractérisée par la présence de quatre cellules périaxiales par segment axial, des synapses secondaires orientées longitudinalement entre les cellules corticales superficielles contigues saillant à l'apex des rameaux, des épaississements lenticulaires dans les parois des cellules médullaires et des rameaux nains caducs pouvant fonctionner comme organes de multiplication végétative (propagules). Cette espèce est aussi caractérisée par l'ensemble de métabolites halogéné suivant: aplysiadiol (diterpénoïde), déoxyprépacifenol (sesquitepénoïde), laurénényne-A and laurénényne-B (C_{15} acétogénines). (Traduit par la Rédaction)

KEY WORDS: Ceramiales, chemotaxonomy, halogenated secondary metabolite, *Laurencia caduciramulosa*, Rhodophyta, vegetative reproduction.

INTRODUCTION

The red algal genus *Laurencia* Lamouroux (Rhodomelaceae, Ceramiales) is second only to *Polysiphonia* in size, containing approximately 135 species that are distri-

buted worldwide, primarily in warmer waters (Masuda *et al.*, 1996). In Vietnam 16 species have been reported (Dawson, 1954b; Pham, 1969; Nguyen *et al.*, 1993), but our Vietnamese expedition during 1992 and 1993 suggests that several undescribed or unrecorded species are additionally present (Suzuki *et al.*, 1995, 1996).

In this paper, we describe a new species which has deciduous branchlets that may function as propagative organs. We also identify halogenated secondary metabolites that characterise this species. As is well documented, species of *Laurencia* produce diverse, unique, halogenated secondary metabolites (Erickson, 1983). Most species of *Laurencia* are characterised by at least one specific secondary metabolite not found in any others (Fenical, 1975; Fenical & Norris, 1975). Other species are characterised by a specific set of secondary metabolites (Masuda *et al.*, 1996). The stability of this specificity and its chemotaxonomic value are supported by Howard *et al.* (1980) and Masuda *et al.* (unpublished results), who observed that the chemistry of several *Laurencia* species remained constant under varying field and culture conditions. Thus, secondary metabolite chemistry can provide criteria for the taxonomy in this troublesome genus (Masuda *et al.*, 1996).

MATERIALS AND METHODS

Specimens for morphological and chemical studies were collected on 7 February 1993 from Hon Tre Island, Tien Hai Islands, Hatien, Kien Giang Province, Vietnam. For morphological studies, specimens were fixed in 4% formalin in seawater, with some later dried as herbarium specimens. Sections were made by hand using a razor blade and pith stick. Tissues were stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1:1:1:1) solution and mounted in 50% glycerol-seawater on microscope slides. Voucher specimens were deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP 062083-062086).

Material for chemical analysis was air-dried at room temperature (about 25° C) for a day. Partially-dried samples (7.8 g) were extracted with methanol, and the methanol solution was concentrated *in vacuo*. The residue was partitioned between ether and water, and the ether solution was shaken with water, dried over anhydrous Na_2SO_4, and then evaporated to leave an oily extract. The methanol extract (272 mg) was fractionated by column chromatography on silica gel (Merck, Kieselgel 60, 70-230 mesh). The fraction (82 mg) eluted with hexane/ethyl acetate (9:1) was further chromatographed by preparative TLC (Merck, Kieselgel $60F_{254S}$) with hexane/ethyl acetate (10:1) to give two fractions. The less polar fraction was subjected to reversed-phase high pressure liquid chromatography (JASCO, Finepak SIL C_{18}; methanol-H_2O [88:12]) to give an inseparable mixture of compounds 3 and 4 (5.3% of the methanol extract). The more polar fraction was re-chromatographed by preparative TLC with toluene to afford compound 2 (2.4%). The fraction (57 mg) eluted with hexane/ethyl acetate (1:1) was further chromatographed by preparative TLC with hexane/ethyl acetate (1:1) to give compound 1 (7.8%). Identification of these compounds was carried out by comparison of the IR and ^1H NMR spectra (400 MHz) as well as by comparing the optical rotations with those of the authentic samples (standards).

RESULTS

Laurencia caduciramulosa Masuda *et* Kawaguchi, sp. nov.

Planta singularis ex axibus rectis multis e disco basali communi et ramis prostratis stoloniformibus effecti constans, purpureo-rubra, aliquantum mollis, exsiccatione chartae firme adhaerens; thalli recti 2-5 cm alti, teretes, axibus principalibus percurrentibus, axes principales usque ad 800 µm in diametro, ramos numerosos in mode irregulariter alterno-spirali ferentes; cellula axialis centralis omnis cum cellulis periaxialibus quattuor; foveae-colligationes secundariae longitudinaliter dispositae inter cellulas corticales superficiales contingentes semper adsunt; cellulae corticales superficiares prope apices ramorum leviter procurrentes, in sectionibus transversalibus ramuli nec radiatim elongatae nec in vallem dispositae; incrassationes lenticulares abundantes in parietibus cellularum medullae; ramuli simplices breves prope apices ramorum adsunt, 100-400 µm longi, 100-160 µm lati ad apicem, constricti ad 70-120 µm latitudines ad basim, caduci intra mm paucas apicum ramorum ferentium; tetrasporangia, cystocarpia et spermatangia non inventa.

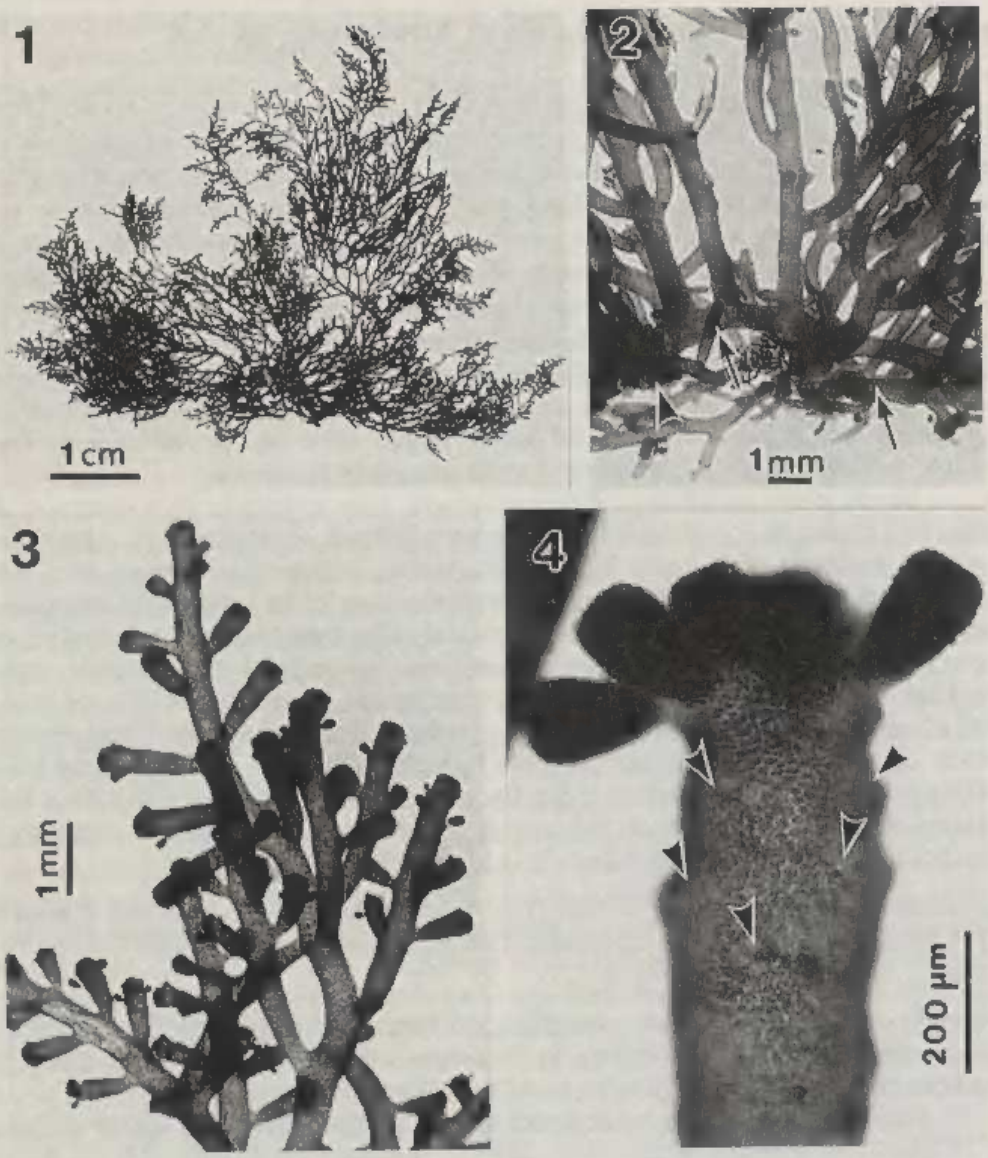
Single plant consisting of many upright axes arising from a common discoid holdfast and stolon-like, prostrate branches, purplish red, somewhat soft, adhering firmly to paper on drying; upright thalli 2-5 cm high, terete, with percurrent main axes, the main axes up to 800 µm in diameter, bearing many branches in an irregularly alternate-spiral manner; each central axial cell with four periaxial cells; longitudinally oriented secondary pit-connections always present between contiguous superficial cortical cells; superficial cortical cells slightly projecting at apices of branches, in transverse sections of branchlets neither elongated radially nor arranged as a palisade; lenticular thickenings abundant in the walls of medullary cells; short, simple branchlets present near apices of branches, 100-400 µm long, 100-160 µm wide at the tip, constricted to 70-120 µm widths at the base, deciduous within a few mm of the apices of the bearing branches; tetrasporangia, cystocarps and spermatangia not found.

Holotype and type locality: SAP 062086 (Fig. 1), collected by M. Masuda & S. Kawaguchi on 7 February 1993, from Hon Tre Island, Tien Hai Islands, Hatien, Kien Giang Province, Vietnam.

Etymology: The specific epithet compounded from *caducus* (dropping off early) and *ramulosus* (bearing branchlets) refers to the characteristic deciduous branchlets that are formed abundantly near branch apices and may function as propagules.

Plants grow on rocks in the lower intertidal zone. Twenty to eighty upright axes (Fig. 1) arise from a common discoid holdfast 2-4 mm in diameter and from stolon-like branches which develop from the lower portions of axes and attach to the substratum secondarily by small discoid holdfasts (Fig. 2). Short descending branches also develop from the proximal portions of upright axes that may function as secondary attachment organs. Upright axes are terete throughout and have main axes which are 500-800 µm in diameter just above the basal disc, 500-600 µm in the lower to middle portions, and taper gradually to 300-400 µm at the uppermost portions.

Many first-order branches are formed in an irregularly alternate-spiral manner 0.5-2 mm apart at angles of 20-40°. These first-order branches are 1.5-3.5 cm long along the lower portions of the main axes and become shorter upwards. They bear progressively shorter branches of up to four orders (Fig. 3), resulting in plants that are pyramidal in shape. Adventitious branches are later produced on the lowest portions of the main axes.



Figs 1-4. *Laurencia caduciramulosa*. Fig. 1. Holotype specimen collected from Hon Tre Island, Tien Hai Islands, Hatien, Kien Giang Province, Vietnam (SAP 062086). Fig. 2. Lower portion of a plant showing stolon-like, prostrate branches (arrows); arrowhead indicates a secondary holdfast. Fig. 3. Upper portion of a second-order lateral. Note some ultimate branches with small deciduous branchlets. Fig. 4. Ultimate branch with small deciduous branchlets; arrowheads indicate scars of shed branchlets.

Small branchlets 100-400 μm long and 100-160 μm wide at the tips develop near branch apices (Figs 3, 4). Their superficial cortical cells are polygonal, not projecting, and are regularly arranged in longitudinal rows in surface view. These superficial cortical cells are 10-16 μm long by 14-20 μm wide in surface view near the apices and 16-30 μm long by 12-16 μm wide proximally. The branchlets are basally constricted, 70-120 μm wide, and fall away readily on slight buffeting, which suggests that they probably function as propagules. Many scars of shed branchlets are present on branches (Fig. 4). Propagule-bearing branches are often recurved. Normal, non-deciduous branches, which are 240-500 μm long and 220-380 μm wide at the tips, are not strongly constricted (Fig. 5), being 180-300 μm wide at the bases.

Apical cells are always immersed in a terminal pit, and central axial cells are recognizable only immediately behind the apical cell (Fig. 6). Each axial cell produces four periaxial cells (Fig. 7). Superficial cortical cells of distal parts of branches of all orders are polygonal, 10-20 μm long by 10-30 μm wide (a length:width ratio of 0.5-1.0), and are regularly arranged in longitudinal rows in surface view. Superficial cortical cells of proximal parts of well-developed branches are 40-100 μm long by 20-36 μm wide (a length:width ratio of 1.5-3.3).

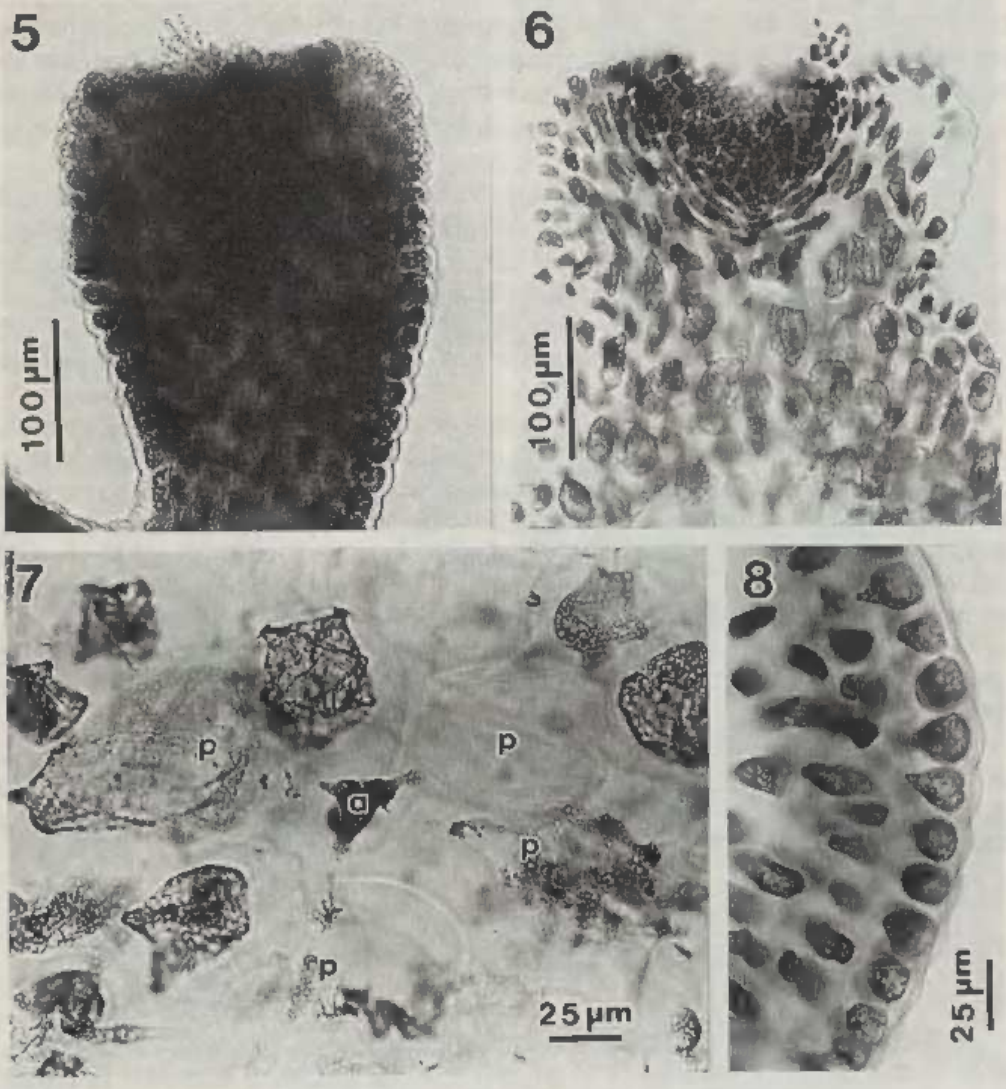
Superficial cortical cells in transverse sections are 16-20 μm thick in the upper portions of first-order branches and 20-50 μm thick in lower portions. They do not form a palisade layer (Fig. 8). Longitudinally oriented secondary pit-connections are present between contiguous superficial cortical cells (Fig. 9). Superficial cortical cells project slightly near branch apices (Fig. 5). Lenticular thickenings are abundant in the walls of medullary cells (Fig. 10) save for the upper portions of branches. Medullary cells are up to 70 μm in diameter in the middle to lower portions of first-order branches.

As no living material is available, examination for the presence of *corps en cerise* was not attempted. Spermatangia, cystocarps and tetrasporangia were not found in our specimens.

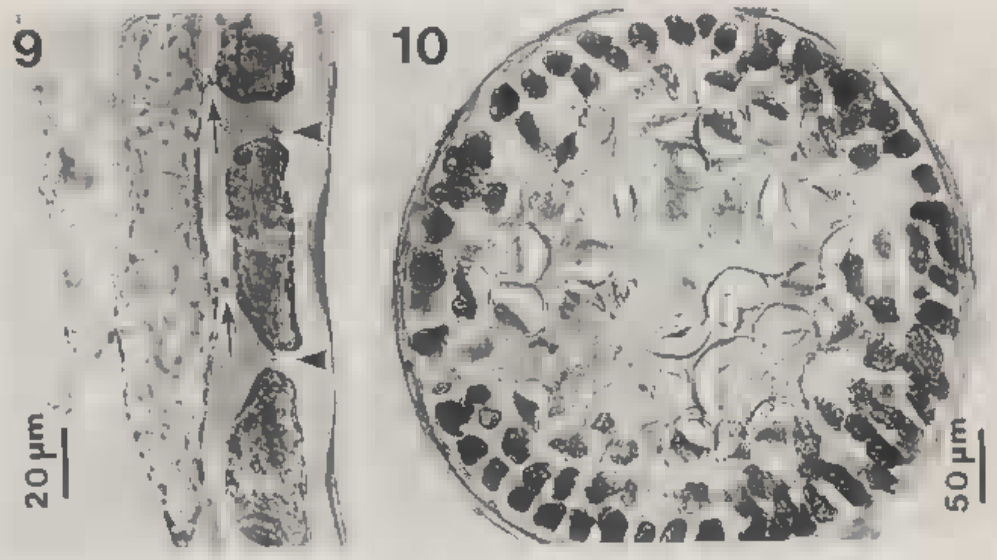
The structural formulae of identified metabolites are shown in Fig. 11. The major metabolite, compound 1, was identified as aplysiadiol, a brominated diterpenoid. Compound 2 was identified as deoxyprepacifenol, a sesquiterpenoid. Compounds 3 and 4 were identified as laurenenyne-A and laurenenyne-B, C_{15} acetogenins.

DISCUSSION

Although no reproductive structures were found, the alga under study is a member of the subgenus *Laurencia* because it has the following features: 1) apical cells always sunk in apical pits; 2) central axial cells recognizable only in close proximity to apical cells; 3) the presence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells; and 4) the production of four periaxial cells per central axial cell (Saito, 1967; Nam *et al.*, 1994; Nam & Sohn, 1994; Nam & Saito, 1995). According to Nam & Saito (1995), the last feature provides a stable basis delimiting the subgenus *Laurencia* from the subgenus *Chondrophycus* Tokida *et* Saito (Saito, 1967), which has two periaxial cells. *Laurencia caduciramulosa* is primarily defined by its deciduous branchlets (possibly function as propagules) and the presence of projecting superficial cortical cells (a feature of relatively few species of *Laurencia* [Masuda & Abe, 1993]). Four species of *Laurencia* have been reported to possess propagules or propagule-like branchlets.



Figs 5-8. *Laurencia caduciramulosa*. Fig. 5. Apex of non-deciduous branch with slightly projecting superficial cortical cells. Fig. 6. Longitudinal section (LS) of a young non-deciduous branch showing the apical pit. Fig. 7. Transverse section (TS) of the upper portion of a second-order branch showing an axial cell (a) with four periaxial cells (p). Fig. 8. TS of the upper portion of a second-order branch.



Figs 9, 10. *Laurencia caduciramulosa*. Fig. 9. LS of middle portion of a second-order branch showing longitudinally oriented secondary pit-connections (arrowheads) between contiguous superficial cortical cells; arrows indicate primary pit-connections. Fig. 10. TS of lower portion of a second-order branch showing abundant lenticular thickenings in the walls of medullary cells.

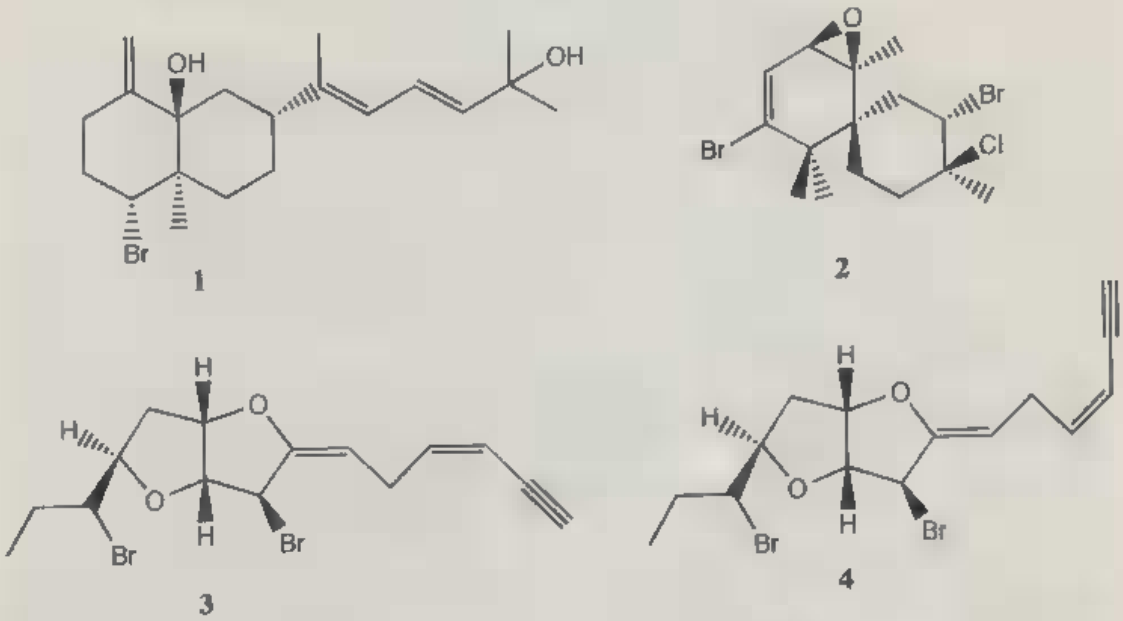


Fig. 11. Molecular structures of secondary metabolites from *Laurencia caduciramulosa*. 1, aplysiadiol; 2, deoxyprepacifenol; 3, laurenenyne-A; 4, laurenenyne-B.

Laurencia gemmifera Harvey (1853), based on material from Key West, Florida, was characterised by having minute bud-like branchlets (Harvey, 1853) and projecting superficial cortical cells (Taylor, 1960; Fujii *et al.*, 1996). However, judging from Harvey's illustration (1853, pl. 18, fig. b), these do not seem to be deciduous. *Laurencia gemmifera* can also be distinguished from *L. caduciramulosa* by its larger thalli (6-8 inches long), thicker axes (as thick as a "crow-quill"), cartilaginous and brittle substance, and patent branches (Harvey, 1853). Furthermore, *L. gemmifera* is entirely different from *L. caduciramulosa* by the production of two periaxial cells from each central axial cell (Fujii *et al.*, 1996). Another tropical Atlantic species, *L. poiteaui* (Lamouroux) Howe, is known to produce propagules (Cruz, Adams & Ballantine, 1996). However, it also produces two periaxial cells from each central axial cell (Fujii *et al.*, 1996). *Laurencia gemmifera* and *L. poiteaui* should be assigned to the subgenus *Chondrophycus*.

Laurencia decidua Dawson (1954a), reported from San Benedicto Island, Revillagigedo Archipelago, Mexico, has deciduous branchlets. However, these branchlets are 1 mm in length (over twice that of *L. caduciramulosa*) and contain tetrasporangia. The Mexican species has no projecting superficial cortical cells. Furthermore, it develops a basal system consisting of densely intergrown, creeping, ramified branches (Dawson, 1954a). *Laurencia caduciramulosa* has no such basal system. The number of periaxial cells per central axial cell for *L. decidua* is unknown.

Laurencia subcorymbosa Dawson (1963), from Cabo Pulmo, Baja California, Mexico, is most similar to *L. caduciramulosa* in having both deciduous propagules and projecting superficial cortical cells, although its number of periaxial cells per central axial cell is unknown. This Mexican species is distinguished from *L. caduciramulosa* by its very slender, sparsely branched axes, those being 200 μm wide below and 300 μm above, and by the absence of stolon-like prostrate branches (Dawson, 1963).

Furthermore, the absence of related species among non-propagulate species of *Laurencia* warrants the establishment of a new species, *L. caduciramulosa*.

More than 250 diverse halogenated or non-halogenated secondary metabolites have been reported from some 30 species of *Laurencia* throughout the world (Erickson, 1983). *Laurencia caduciramulosa* is characterised by the following set of halogenated secondary metabolites: aplysiadiol (diterpenoid), deoxyprepacifenol (sesquiterpenoid), laurenenyne-A and laurenenyne-B (C_{15} acetogenins). Although aplysiadiol is first reported from algal species in the present study, it has been isolated from a sea hare *Aplysia kurodai* (Baba) collected at Shima Peninsula, Mie Prefecture, Japan (Ojika *et al.*, 1990). This suggests that the sea hare consumes *Laurencia* species producing aplysiadiol. Various species of sea hares are said to consume large quantities of algae including *Laurencia* and concentrate the algal metabolites in their digestive gland (Fenical, 1975). The halogen-containing components appear to suffer little degradation and diffuse throughout the animal, and may be effective against predators (Fenical, 1975). Deoxyprepacifenol has previously been obtained from a sea hare *Aplysia californica* Cooper collected at La Jolla, California, U.S.A. (Ireland *et al.*, 1976), and two species of *Laurencia* growing in Japan, *L. okamurae* Yamada (Ojika *et al.*, 1982; Suzuki & Kurosawa, 1985) and *L. nipponica* Yamada (Kikuchi *et al.*, 1985; Suzuki *et al.*, 1985). Laurenenyne-A and laurenenyne-B have been isolated from an undescribed species of *Laurencia* collected at Kamishima, Toba, Mie Prefecture, Japan (Suzuki *et al.*, 1993). Species of *Laurencia* that produce a set of these compounds are not reported. Halogenated secondary metabolites of *L. decidua* have been reported in material from Baja California, Mexico (McMillan *et al.*, 1976). These compounds and those of *L. caduciramulosa* do not overlap with each other.

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